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Single neuron responses in humans during execution and observation of actions

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Summary

Go to:

Direct recordings in monkeys have demonstrated that neurons in frontal and parietal areas discharge during execution and perception of actions [1-8]. Since these discharges ‘reflect’ the perceptual aspects of actions of others onto the motor repertoire of the perceiver, these cells have been called *mirror neurons*. Their overlapping sensory-motor representations have been implicated in observational learning and imitation, two important forms of learning [9]. In humans, indirect measures of neural activity support the existence of sensory-motor mirroring mechanisms in homologue frontal and parietal areas [10, 11], other motor regions [12-15], and also the existence of multi-sensory mirroring mechanisms in non-motor regions [16-19]. We recorded extracellular activity from 1177 cells in human medial frontal and temporal cortices while patients executed or observed hand grasping actions and facial emotional expressions. A significant proportion of neurons in supplementary motor area, and hippocampus and environs, responded to both observation and execution of these actions. A subset of these neurons demonstrated excitation during action-execution and inhibition during action-observation. These findings suggest that multiple systems in humans may be endowed with neural mechanisms of mirroring for both the integration and differentiation of perceptual and motor aspects of actions performed by self and others.

Highlights

Go to:

1. Cells in SMA respond during execution and observation of actions
2. Cells in medial temporal lobe respond during observation & execution of actions
3. Some respond with excitation during execution and inhibition during observation

Results

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We recorded extracellular activity from a total of 1177 neurons in 21 patients while they observed and executed grasping actions and facial gestures. In the observation conditions, subjects observed various actions presented on a laptop screen. In the execution conditions, the subjects were cued to perform an action by a

visually presented word. In a control task, the same words were presented and the patients were instructed not to execute the action (see Experimental procedures and figure S1A). In the medial frontal cortex, we recorded from 652 neurons (369 single units, and 283 multi units) in the supplementary motor area (SMA; both SMA-proper and pre-SMA), and anterior cingulate cortex (ACC; both the dorsal and rostral aspects [20]). In the medial temporal lobe we recorded from 525 neurons (296 single units, and 229 multi units) in the amygdala, hippocampus, parahippocampal gyrus (PHG) and entorhinal cortex (EC) (see figure S1B for anatomical location of electrodes). The number of cells recorded in each region is provided in Table 1A.

Table 1A
Location of recorded cells **A)** Number of single units (SU) and multi units (MU) recorded in the left and right hemispheres in various anatomical regions.

Significant changes in firing rate were tested using two-tailed paired t-test between the firing rate during baseline (−1000 ms to 0 ms relative to trial onset) and a window of +200 to +1200ms after stimulus onset (see Experimental procedures). For each action (smile, frown, precision grip, or wholehand grip) we examined the neural response during action-observation and action-execution. A response to action-execution was considered only if there was no significant response to the corresponding control task.

After examining the cell's response to each action separately, the cell was classified as follows:

Action-observation neuron

a cell responding only during one or more action-observation conditions and not during any of the action-execution conditions (e.g. a cell responding to smile observation and frown observation).

Action-execution neuron

a cell responding only during one or more action-execution conditions and not during any of the action-observation conditions (e.g. a cell responding to precision-grip execution).

Action Observation/Execution non matching neuron

a cell responding during action-observation in one condition and action-execution in a different condition (e.g. a cell responding to smile observation and frown execution).

Action Observation/Execution matching neuron

a cell responding during both the execution and the observation of the same action (e.g. a cell responding to smile observation and smile execution).

Table 1B provides the number of cells in each category described above, according to anatomical region. The majority of cells responded to one dimension of the stimuli (observation or execution). In the SMA ($\chi^2(1) = 14.5$, $p = 10^{-3}$) and pre-SMA ($\chi^2(1) = 4.2$, $p = 0.03$), the proportion of responses to action-execution relative to action-observation was significantly higher. In the other regions examined (ACC and medial temporal lobe) there was no significant difference between the two conditions. Six cells responded to observation, execution, and also the control task of one action and were therefore not considered as *Action Observation/Execution matching cells* (three cells in PHG, two in EC and one in SMA). Within the population of *action-observation* cells, there were more responses to hand grasps (precision grip or wholehand prehension) in PHG relative to facial gestures (smile or frown; ($\chi^2(1) = 3.9$, $p = 0.04$)), and more responses to observations of facial gestures relative to hand grasps in ACCd ($\chi^2(1) = 4.8$, $p = 0.02$). The distribution of responses within the population of *action-observation* and *action-execution* cells is provided in Table S1.

Table 1B
B) Response types of cells across all recorded regions. Absolute number (single unit, multi unit) and percentages of cells (calculated from

total number of recorded cells in each region – see table 1A). Last column represents the percentage of ...

We subsequently focused our analyses on the Action Observation/Execution Matching cells responding during both observation and execution of particular actions. Figure 1A displays one such cell in the SMA responding to the observation and execution of two grip types (Precision and Wholehand). This cell did not respond to the Control tasks or any of the facial gesture conditions. Figure 1B displays another cell in entorhinal cortex responding to observation and execution of facial gestures (Smile and Frown). Again, this cell did not respond to the Control tasks or to observation and execution of the various Grips.

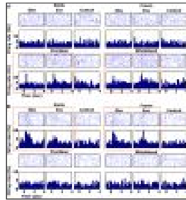

Figure 1
Rasters (top) and peri-stimulus time histograms (bottom) of two cells during all experimental conditions and tasks. Rasters are aligned to stimulus onset (red vertical line at time = 0). Bin size = 200ms. Red box highlights responses passing statistical ...

Next, we tested whether the proportion of Action Observation/Execution matching neurons in each anatomical region is significantly higher than that expected by chance (chance level set at 5%). We performed a chi-square test on the proportion of such cells in each region (except for the amygdala where we performed Fischer's exact test due to small number of cells). The proportion of cells in the hippocampus ($\chi^2(1) = 12.5$, $p = 2\times10^{-4}$), parahippocampal gyrus ($\chi^2(1) = 17.4$, $p < 10^{-4}$), entorhinal cortex ($\chi^2(1) = 3.3$, $p < 0.05$), and SMA ($\chi^2(1) = 19.4$, $p < 10^{-4}$) was significantly higher than expected by chance. In Amygdala, pre-SMA, ACCd and ACCr the proportions were not significantly higher than chance. In addition to the chi-square test, we performed a bootstrap analysis to test whether or not the number of Action Observation/Execution matching neurons is higher than the null distribution (see Experimental procedures). Figure S2A displays the null distribution (blue) together with the actual number of cells in our data set (red arrow). In agreement with the chi-square test described above, the number of cells in SMA ($p = 0.003$), entorhinal cortex ($p = 0.001$), hippocampus ($p < 10^{-4}$), and parahippocampal gyrus ($p < 10^{-4}$) were significantly higher than expected by chance. In addition, we performed the same analysis, this time taking into account only cells defined as single units and obtained similar results (SMA ($p = 0.02$), EC ($p = 0.004$), H ($p = 0.02$) and PHG ($p = 0.007$); see figure S2B). Furthermore, the proportion of Action Observation/Execution matching neurons in these regions was significantly higher compared with Poisson generated spike trains with similar firing rates (figure S2C). The distribution of joint p-values for these Action Observation/Execution matching neurons is provided in figure S2D for the different regions.

Next, we focused on the Action Observation/Execution matching neurons in the anatomical regions where the proportion of such cells was significant (SMA, parahippocampal gyrus, hippocampus, and entorhinal cortex). Figure 2 displays the responses of six additional neurons from these various regions. The complete response details of all Action Observation/Execution Matching cells are provided in Table S2. The majority of these cells (40 out of 68) were classified as single units (see Experimental procedures). Among the 68 Action Observation/Execution Matching cells, 33 increased their firing rate during both observation and execution of a particular action (e.g. Figure 2 A-D). In contrast, 21 other neurons decreased their firing rate during both conditions (Figure 2E). These types of responses have been previously reported in monkeys (e.g. [21]) and birds [22]. Furthermore, 14 neurons *increased* their firing rate during one condition and *decreased* it during the other. The majority of these cells ($n = 11$) increased their firing rate during action-execution and decreased their firing rate during action-observation (Figure 2F) while the remaining neurons did the opposite ($\chi^2(1) = 6.2$, $p = 0.01$). For anatomical distribution of response types see Table S3A. Obviously, the breaking down of responses by type and anatomical region makes it difficult to test for regional differences and therefore to draw any firm conclusion on these distributions.

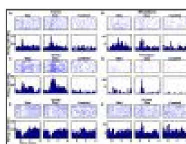

Figure 2
Raster plots and peri-stimulus time histograms of 6 different observation/execution matching neurons during execution, observation, and the control task. (A) Single unit in left entorhinal cortex increasing its firing rate during both frown execution ...

We subsequently examined the temporal profiles of neural activity by computing the average response profile of all Action Observation/Execution matching neurons. This was conducted separately for cells exhibiting excitation to both conditions (Figure 3A), inhibition to both conditions (Figure 3B), and cells exhibiting excitation during action-execution and inhibition during action-observation (Figure 3C). In order to accommodate for differences in firing rates across different cells before averaging, similar to [21] we normalized each excitatory response to range between 0 and +1, and each inhibitory responses to range between 0 and −1 (see Experimental procedures). Excitatory cells reached peak firing rate faster during action-observation compared with action-execution and inhibitory cells returned to baseline faster during action-observation. It is interesting to note that excitatory observation/execution matching cells had firing rates significantly lower than baseline during the control task (Figure 3A). Average baseline firing rates for cells exhibiting excitation during both action-execution and action-observation was 4.8±3.7Hz, whereas the average baseline firing rates for cells exhibiting inhibition during both conditions was 9.4±6.0Hz (mean ± s.d.). Average baseline firing rate for cells exhibiting excitation to action-execution and inhibition to action-observation was 6.5±3.2. For relative and absolute response amplitudes see figure S3. In terms of response latencies, no significant difference between observation and execution was found (see table S3B).

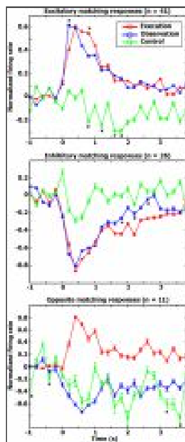

Figure 3
Average normalized response profile of all Action Observation/Execution matching neurons. (A) Average of 41 excitatory responses (from 33 different neurons) during action execution and action observation. (B) Average of 26 inhibitory response (from 21 ...

The majority of Action Observation/Execution matching neurons in our data-set matched only one action (54 cells), while 14 cells matched the execution and observation of two actions. No significant difference between the proportion of cells matching facial gestures or hand grasps was found ($\chi^2(1) = 0.6$, $p = 0.4$; see Table S1C).

Discussion

Go to:

We recorded extra-cellular neural activity in 21 patients while they executed and observed facial emotional expressions and hand grasping actions. In agreement with the known motor properties of SMA and pre-SMA, our results show a significantly higher proportion of cells responding during action-execution compared with action-observation in these regions. While the majority of responding cells across all regions responded only to one aspect of a particular action (either perception or execution) we also found cells responding to both. Significant proportions of such cells were found both in medial frontal lobe (SMA) and in medial temporal lobe – namely, hippocampus, parahippocampal gyrus and entorhinal cortex. In the amygdala, ACC (both rostral and dorsal aspects), and pre-SMA, the number of such cells did not reach significance levels. Finally, within the population of cells responding to both observation and execution of action, our results indicate a sub-population of cells responding with excitation during action-execution and inhibition during action-observation.

What is the relationship between the cells recorded in SMA – on the medial wall of the frontal lobe - that responded during both execution and observation of actions and the 'mirror neurons' reported previously in monkeys? The critical feature of mirror neurons is the functional matching between a motor response and a perceptual one [23]. The population of cells we found exhibited this critical functional feature for grasping actions and facial expressions. In this regard, there is obviously similarity between the human and the monkey cells. In monkeys, however, neurons with mirroring properties have been reported in a variety of areas on the lateral wall of the primate brain [3, 7, 21, 24, 25]. In the current study we did not record from these regions since the placement of electrodes was determined only by clinical considerations. Neurophysiological data

suggest that while areas on the lateral wall such as F5 seem to contain a vocabulary of actions, from grasping to facial expressions, areas on the medial wall such as SMA seem relevant to movement initiation and movement sequences [26]. Thus, it is possible that the action observation/execution matching neurons we recorded from SMA represent cellular mirror mechanisms for these particular aspects of hand and facial actions.

One of the striking features of our findings is the presence of action observation/execution matching neurons in the medial temporal lobe (MTL). Connections such as the uncinate fasciculus and other cortico-cortical white matter tracts between the MTL and motor regions in the frontal lobe exist [27-31]. Although there is some evidence for responses in the hippocampus during voluntary actions [32], unlike SMA, lesions in the medial temporal lobe do not result in obvious motor deficits, and electrical stimulation in these areas does not result in overt movement. It might be argued that the visual input (rather than the motor output) during action-execution is what elicited the responses in these medial temporal lobe neurons. In our study, however, the visual inputs during action-observation and action-execution were widely different (only a word is visually presented to cue action-execution compared to a video/picture presented during action-observation). Furthermore, the visual input during the control and action-execution of the face experiment is identical although these cells did not respond to the control condition (see figures 1-2). Additionally, in some patients we used auditory tones to cue action-execution (and as appropriate control) and we obtained similar results for these patients (i.e. responses to the tone during action-execution and not during the control condition). It follows that the purely visual explanation for action observation/execution matching cells cannot hold, at least for the execution of facial expressions where no additional visual input is available. In principle, the visual input of the patient's grasping hand may explain the discharge of the cells during grasping execution and grasping observation. However, this argument would require two separate mechanisms to explain the mirroring responses for facial expressions and for grasping: a ‘true’ mirroring mechanism for facial expression and a ‘purely visual’ mechanism for grasping action. While this possibility cannot be excluded, it is less parsimonious than invoking a unitary mirroring mechanism for both facial expressions and grasping actions.

It may also be argued that the neurons with mirroring properties respond in an invariant manner to different visual stimuli sharing the same concept e.g., a picture of a smiling face and the execution cue word “smile” [33]. Indeed we found six neurons that responded to observation, execution and also to the control condition of a specific action. However the argument that the observation/execution matching neurons in the medial temporal lobe represent the concept of the action is untenable since we only considered cells that did not respond during the control conditions where the word stimuli were presented again but did not cue the patient to perform an action. An alternative account for the responses in medial temporal lobe during action-execution is that they represent proprioceptive processing. At this stage we cannot rule out this alternative account.

We have recently demonstrated that neurons in medial temporal lobe are re-activated during spontaneous recall of episodic memory [34]. The action observation/execution matching neurons in the medial temporal lobe may match the sight of actions of others with the memory of those same actions performed by the observer. Thus during action-execution, a memory of the executed action is formed, and during action-observation this memory trace is reactivated. This interpretation is in line with the hypothesis of multiple mirroring mechanisms in the primate brain, a hypothesis that can easily account for the presence of mirroring cells in many cortical areas [1, 3-5, 7, 8, 24, 25].

The functional significance of the mirror mechanism most likely varies according to the location of mirror neurons in different brain areas [35]. For example, the mirror mechanism in the insula might underlie the capacity to understand a specific emotion (disgust) in others [16, 19], while the mirror mechanism in the parieto-frontal circuit may help understanding the goal of observed motor acts and the intentions behind them [21]. Here we show cellular mirroring mechanisms in areas relevant to movement initiation and sequencing (SMA), and memory (medial temporal lobe). While these hypothesis have yet to be tested more carefully, these results demonstrate the presence of mirror mechanisms in humans at the single neuron level and in areas functionally different from the ones previously described in the literature.

Mirroring activity, by definition, generalizes across agency and matches executed actions performed by self with perceived action performed by others. While this may facilitate imitative learning, it may also induce unwanted imitation. Thus, it seems necessary to implement neuronal mechanisms of control. The subset of mirror neurons responding with *opposite* patterns of excitation and inhibition during action-execution and

action-observation seem ideally suited for this control function. Indeed, extensive brain lesions are associated with compulsory imitative behavior in neurological patients [36, 37]. Recently, it has been reported that the majority of pyramidal tract neurons in monkey F5 that display mirror like activity suppress their firing rate during action observation [6], in accord with our own data. Interestingly, some fMRI studies have also reported decreased BOLD signal in primary motor cortex during action-observation [12]. A recent model proposes a direct mirror pathway for automatic, reflexive imitation and an indirect mirror pathway for parsing, storing and organizing motor representations [38]. The observation/execution matching cells with opposite response patterns are compatible with the direct pathway. Finally, mirroring may generate the problem of differentiating between actions of the self and of other people. The opposing pattern of activity for actions of self and others may also form a simple neuronal mechanism for maintaining self-other differentiation.

To conclude, these data demonstrate mirroring spiking activity during action-execution and action-observation in human medial frontal cortex and human medial temporal cortex - two neural systems where mirroring responses at single cell level have not been previously recorded. A subset of these mirroring cells exhibited opposing pattern of excitation and inhibition during action-execution and action-observation, a neural feature that may help preserving the sense of being the owner of an action during execution, and exert control on unwanted imitation during observation. Taken together, these findings suggest the existence of multiple systems in the human brain endowed with neural mirroring mechanisms for flexible integration and differentiation of the perceptual and motor aspects of actions performed by self and others.

Experimental Procedures

Go to:

For detailed description of methods see Supplemental Experimental Procedures.

Patients

Go to:

We recorded extracellular single and multi unit activity from 21 patients with pharmacologically intractable epilepsy. Patients were implanted with intracranial depth electrodes to identify seizure foci for potential surgical treatment. Electrode location was based solely on clinical criteria and the patients provided written informed consent to participate in the experiments. The study conformed to the guidelines and was approved by the Medical Institutional Review Board at UCLA.

Experiment design

Go to:

The entire experiment was composed of three parts – '*Facial expressions*', '*Grasping*' and a '*Control'* experiment. Stimuli were presented on a standard laptop at the patient's bed. In the 'Grasping' experiment there were two conditions: action-observation, and action-execution. In the action-observation conditions, the subjects observed a 3sec video clip depicting a hand grasping a mug with either precision grip or whole hand prehension. In the action-execution condition, the word 'Finger' appearing on the screen cued the subject to perform a precision grip on a mug placed next to the laptop. Similarly, the word 'Hand' cued the subject to perform a whole hand prehension. Observation and execution trials were randomly mixed. The 'Facial expressions' experiment was also composed of execution and observation trials. In the execution trials, the subjects smiled or frowned whenever the word 'Smile' or 'Frown' respectively appeared on the screen. In the observation conditions they simply observed an image of a smiling or frowning face. Observation and execution trials were randomly mixed. In the 'Control' experiment, the subjects were presented with the same cue words used in the execution conditions of the 'Facial expressions' and 'Grasping' experiments (i.e. the words 'Finger', 'Hand', 'Smile', or 'Frown'). This time, the subjects had to covertly read the word and refrain from making facial gestures or hand movements.

Recording and analysis

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Data was recorded at 28kHz using a 64-channel acquisition system (Neuralynx, Tucson, AZ) and the signals were band-pass filtered between 1Hz and 9kHz. During off-line analysis, the raw signal was band-pass filtered between 300 and 3000 Hz and action potentials were clustered and manually sorted using an algorithm based on super paramagnetic clustering. For each neuron, and each condition, we assessed responsiveness by comparing the firing rate during baseline (−1000ms to 0ms relative to stimulus onset) and firing rate during the experimental condition (+200ms to +1200ms relative to stimulus onset) on a trial by trial basis using a two-tailed paired t-test. The statistical significance threshold for the paired t-test across trials was set at 0.05. In

order to calculate the average response profile of cells during execution/observation (Figure 3), Excitatory responses were normalized by subtracting the average response during baseline (−1000 to 0ms relative to trial onset), and dividing by the maximum firing rate of the response (bin size = 200ms). Inhibitory responses were normalized by removing the average response during baseline and dividing by the absolute value of the minimum of the response (see Supplemental Experimental Procedures for further details).

Supplementary Material

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Supplementary online material for

'Single neuron responses in humans during execution and observation of actions'

Mukamel, Ekstrom, Kaplan, Iacoboni, and Fried

Figure S1 (related to Figure 1): Experimental design. **A**) The experiment was composed of three parts – *Grasp, Facial expressions* and *Control*. During *Grasp*, subjects were presented with video clips of a hand grasping a mug and with the words 'Finger' or 'Hand'. They were instructed to grasp a mug with precision grip or whole hand prehension when the words 'Finger' or 'Hand', respectively, were presented and to simply observe when the video clips were played. During *Facial expressions*, subjects were presented with a picture of a smiling or a frowning face and with the word 'Smile' or 'Frown'. They were instructed to perform the corresponding action when the words were presented and to simply observe when the pictures were presented. Subjects were also instructed to refrain from making any hand movements or facial gestures during all observation conditions. One experimenter always supervised subject's compliance during the tasks. In the *Control* task, subjects were presented with the words used as cues in the *Grasp* and *Facial expression* parts of the experiment and were instructed to covertly read the words and refrain from making hand movements or facial gestures. **B**) Anatomical location of electrodes in all 21 patients. Electrode location in each patient was verified by co-registering the post-operative CT scan with the pre-operative structural MRI. Electrode positions were transformed into MNI space and are presented on the MNI 305 brain. Top row shows the electrodes in the medial frontal lobe and bottom row displays the electrodes in the medial temporal lobe. LH – left hemisphere, RH – right hemisphere; A – anterior, P – posterior; SMA – supplementary motor area; ACCd – dorsal aspect of anterior cingulate cortex, ACCr – rostral aspect of anterior cingulate; A – amygdala, PHG – parahippocampal gyrus, H – hippocampus, EC – entorhinal cortex.

Figure S2 (related to Figure 2): **A**) Bootstrap analysis. In order to assess if the proportion of Action observation/execution matching neurons is significant or not, we compared the actual number of Action observation/execution matching neurons in each region (red arrow) with the null distribution computed over 10,000 iterations (blue bars; see methods). The vertical red line represents the 5% chance level. Note that the number of cells in SMA, hippocampus, parahippocampal gyrus, and entorhinal cortex was significantly higher than expected by chance. **B**) Same analysis as described for panel A but only using data recorded from single units (as opposed to single and multi units used in panel A). Again, the number of Action observation/execution matching cells in SMA, H, PHG and EC was higher than in the shuffled data at significance level of $p < 0.05$. **C)** Number of Action observation/execution matching neurons compared with Poisson generated spike trains with similar firing rates. For each recorded neuron, we calculated the average firing rate and generated surrogate spike trains with Poisson distributed inter-spike intervals and similar firing rate. Next, we assessed whether the neuron with the surrogate spike trains would be considered an Action observation/Execution matching cell. This was performed for each neuron in the population and the number of pseudo action observation/execution matching cells was counted. The blue columns show the distribution of number of action observation/execution matching neurons in the surrogate data after 10,000 iterations. The red arrow points to the actual number of action observation/execution matching cells in the real data. The red vertical line represents 1% chance level. **D**) P-value of response during action-execution (x axis) and action-observation (y-axis) for all action observation/execution matching cells. Acronyms for anatomic regions as in Figure S1.

Figure S3 (related to Figure 3): Scatter plots of response amplitude during action-observation and action-execution. (A) For each neuron, the firing rate during action-execution was divided by the firing rate during baseline (x-axis). Similarly, the firing rate during action-observation was divided by the firing rate during baseline (y-axis). Green circles – cells exhibiting excitation to both conditions; Black circles – cells exhibiting inhibition during both conditions; Blue circles – cells exhibiting excitation during action execution and inhibition during action observation; Red circles – cells exhibiting excitation during action-observation and inhibition during action-execution. (B) Absolute firing rates of the same cells shown in (A).

Table S1 (related to Table 1): Distribution of cells responding during action-execution (A) and action-observation (B) in the different anatomical regions. Face – number of cells responding during execution (observation) of a facial gesture (smile or frown); Hand – number of cells responding during execution (observation) of a hand grip (precision grip or wholehand prehension); Both – number of cells responding during execution (observation) of a facial gesture and also a hand grip. Within the population of cells responding during action-observation, the proportion of cells responding to observation of hand grasps in PHG was significantly larger than those responding to observation of facial gestures ($\chi^2(1) = 3.9$, $p = 0.04$). The proportion of cells responding to observation of facial gestures in ACCd was significantly larger than the proportion of cells responding to observation of hand grasps ($\chi^2(1) = 4.8$, $p = 0.02$). **C**) Anatomical distribution of Action observation/execution matching cells for the different conditions (Smile, Frown, Precision grip, and Wholehand prehension). Other, refers to 14 cells matching more than one condition. Six of those cells matched both facial gestures (Smile and Frown; One cell in H, one in PHG, one in EC and three in SMA). Four cells matched both hand grasps (Precision and Wholehand; One in SMA, one in EC, and two in PHG). The remaining four cells matched one facial gesture, and one hand grasp (two cells in EC and one in SMA matched 'Frown' and 'Precision'; one cell in SMA matched 'Smile' and 'Wholehand').

Table S2 (related to Table 1): Response details of all cells matching execution/observation. Column 1: Serial number of cell. Column 2 (Region): first letter (L or R) corresponds to the hemisphere from which the cell was recorded (Left or Right). Columns 3 – 6 (Execution): letters correspond to the different conditions (S = 'Smile', F = 'Frown', P = 'Precision', and W = 'Wholehand'). Conditions are in descending order of response magnitude (firing rate). Thus for excitatory responses, firing rate in column 3 > firing rate in column 6 and for inhibitory responses firing rate in column 3 < firing rate in column 6. Red letters correspond to significant difference in firing rate relative to baseline and asterisks denote significant difference in firing rate relative to the corresponding control condition. Minus signs denote significant difference of response in columns 4, 5, and 6 relative to condition in column 3. Columns 7 – 10 (Observation): same as columns 3 – 6 but for the observation condition. Column 11 (Control): significant responses (if any) to control conditions (letters same as in columns 3 – 10). Column 12 (Response type): Excitatory (E), Inhibitory (I), or Both (B representing excitation to execution and inhibition to observation and B excitation to observation and inhibition to execution). Column 13 (Congruency): Broadly congruent cells (B) and Matching cells (M) – see supplemental experimental procedures for definition). Column 14 (Unit type): Single unit (SU) vs. Multi unit (MU).

Table S3 (related to Figure 3): **A**) Anatomical distribution of responses of Observation/Execution matching cells. Top row, number of cells (single, multi units) responding with excitation during both action-execution and action-observation. Middle row, number of cells responding with inhibition to both conditions. Bottom row, cells responding with excitation during action-execution and inhibition during action-observation. Two additional cells in PHG responded with excitation during action-observation and inhibition during action-execution. One more cell in EC responded to two conditions (inhibition to frown-execution and excitation to frown-observation; excitation to precision-execution and inhibition to precision-observation). Regional acronyms as in Table 1. **B, C)** Latencies (in ms) of excitatory (**B**) and inhibitory (**C**) action observation/execution matching cells. Latencies were computed as the time between stimulus onset and the first time bin at which neural response reached maximum/minimum value (bin size 100ms). Within each region, no statistical difference between observation and execution latencies was found (two-tailed, paired t-test across all cells). We also compared the latency of SMA neurons with all other temporal lobe regions. The SMA excitatory responses had shorter latencies during action-execution compared with the hippocampus ($p = 0.03$, two-sample equal variance t-test).

Supplemental Experimental Procedures

Patients and experimental setup

We recorded extracellular single and multi unit activity from patients with pharmacologically intractable epilepsy, implanted with intracranial depth electrodes to identify seizure foci for potential surgical treatment. Data was acquired from 21 patients in 43 sessions (range 1 to 5 sessions per patient; median = 2). Mean patient age was 31 (range 18 – 54); 12 males; 15 right handers. Electrode location was based solely on clinical criteria. Each electrode terminated in a set of nine 40-μm platinum-iridium microwires and the signals from eight micro-electrodes were referenced to the ninth, lower impedance micro-electrode [1]. Data was recorded at 28kHz using a 64-channel acquisition system (Neuralynx, Tucson, AZ) and the signals were band-pass filtered between 1Hz and 9kHz. The beginning and end of each experimental trial was marked by electrical triggers sent from the laptop to the recording device. Patients provided written informed consent to participate in the experiments. The study conformed to the guidelines and was approved by the Medical Institutional Review Board at UCLA.

Experiment design

The entire experiment was composed of three parts – '*Facial expressions*', '*Grasping*' and a '*Control'* experiment. Order of experimental parts was randomized across subjects. Stimuli were presented on a standard laptop at the patient's bed. In the case of the grasping part of the experiment, a mug was placed next to the laptop.

Facial expressions

Patients were presented with pictures of smiling or frowning faces, or with the written word 'Smile' or 'Frown'. The patients were instructed to simply observe the picture and avoid making any facial gestures, but to perform the facial gesture when the written word smile or frown was presented. The experiment started with 6 seconds of a blank grey screen. The pictures/text instructions were presented for one second and followed by a blank grey screen which lasted either 5 or 6 seconds randomly. Pictures of 16 different individual faces were presented (8 male and 8 female faces) in either a smiling or frowning configuration (total of 32 different images). Each individual image was presented once thus there were 16 trials for smile observation and 16 trials for frown observation. Similarly there were 32 facial gesture execution trials (16 'smile', and 16 'frown'). The order of trials was counter balanced. Total duration for this part was 7:08 minutes.

Grasp

The patients were presented either with 3 second video clips depicting a hand grasping a mug or with the written word 'Finger' or 'Hand'. They were instructed to observe the video clip and refrain from making any hand movements. The video clips depicted a hand grasping a mug with either precision grip or whole-hand grasp. When written words were presented, the patients performed a precision grip (for the word 'Finger') or a wholehand grasp (for the word 'Hand') on a mug placed next to the laptop. The patients performed 36 observation trials (18 trials of precision grasps and 18 trials of whole hand grasps) and 36 execution trials (18 'Finger' and 18 'Hand'). Each trial was followed by a blank grey screen lasting either 5 or 6 seconds. The order of trials was counterbalanced. This part of the experiment lasted 9:12 minutes.

Control

The patients were presented with a written word for one second (either 'Smile', 'Frown', 'Finger' or 'Hand'). These words were the ones used as cues for action-execution in the previously described parts of the experiment (*Facial expression* and *Grasp*). Each word presentation was followed by a blank grey screen lasting either 5 or 6 seconds randomly. Patients were instructed to covertly read the words and refrain from making hand movements/facial gestures. This part of the experiment, lasting 3:40 minutes, was composed of 32 trials (8 trials for each word) presented in a counterbalanced fashion.

The first five patients performed a variation of the task. In the execution conditions of the *Facial expression* and *Grasp* parts of the experiment, instead of a word appearing on the screen to cue the patient to perform the appropriate action, a 100 millisecond auditory tone was used. A low tone (250Hz) cued the patient to frown or perform a whole hand prehension in the *Facial expression* and *Grasp*

experiments respectively. Similarly, a high tone (1000Hz) indicated to smile or perform a precision grasp. In the control experiment the same tones were played but the patients were explicitly instructed to simply listen to the tones and avoid making any facial gestures or hand movements during the experiment. There were a total of 24 hand execution trials (12 precision grasp, 12 wholehand prehension), and similarly another 24 hand observation trials. In the *Facial expression* experiment there were 32 observation trials (16 smiling, 16 frowning) and 24 execution conditions (12 smiling, 12 frowning). The execution and observation conditions were separated into blocks and the patients were notified in advance if it was an observation block or an execution block. In the *Control* experiment, each beep was sounded 5 times in a counterbalanced fashion. In order to simplify the task for the patients, the remaining 16 patients were explicitly cued for action execution using written words as described above. Our analyses did not reveal differences between the responses of the first five patients and of the remaining 16 patients. Thus, data from both groups are collapsed here.

Data analysis

Anatomical Localizations

In order to verify the position of implanted electrodes, CT scans following electrode implantation were co-registered to the preoperative MRI using Vitrea® (Vital Images Inc.). In the frontal lobe, we recorded from 16 different patients in rostral ACC, 7 patients in dorsal ACC, 6 patients in pre-SMA, and 5 patients in SMA. In the temporal lobe, we recorded from 4 patients in the Amygdala, 15 patients in the Hippocampus, 7 patients in entorhinal cortex, and 12 patients in the parahippocampal gyrus.

Spiking activity

The raw signal was band-pass filtered between 300 and 3000 Hz and a threshold of five standard deviations above the median of the filtered signal was used to detect suspected action potentials. The suspected action potentials were then clustered and manually sorted as spikes or electrical noise [2]. Similar to [3], the classification between single unit and multi-unit was done visually based on the following: (1) Average spike shape and its variance; (2) the ratio between the spike peak value and the noise level; (3) the inter-spike interval distribution of each cluster; and (4) the presence of a refractory period for the single units (that is, less than 1% of spikes within less than 3ms inter-spike interval).

Statistical analysis

For each neuron, and each condition, we assessed responsiveness by comparing the firing rate during baseline (−1000ms to 0ms relative to stimulus onset) and firing rate during the experimental condition (+200ms to +1200ms relative to stimulus onset) on a trial by trial basis using a two-tailed paired t-test. The statistical significance threshold for the paired t-test across trials was set at 0.05.

Bootstrap analysis (figures S3, S4)

In order to assess whether or not the number of Action Observation/Execution matching neurons in each region is significant, we did the following. For each neuron recorded in a given region (regardless of responsiveness), we shuffled the spike trains from each trial across the different conditions. Thus, in the new shuffled data-set each individual spike-train is real but it is assigned randomly to the different conditions (e.g. a spike-train originally recorded during smile-execution will be assigned to precision-grip observation in the shuffled data-set). Next, we assessed whether or not the neuron would be considered an Action Observation/Execution matching neuron using the same criteria we used for the original data. This was performed for all recorded cells in a given anatomical region and the proportion of pseudo Action Observation/Execution matching neurons was computed. In order to calculate the null distribution, this procedure was repeated for 10,000 iterations. Figures S3, and S4 display the distribution of proportions of Action Observation/Execution matching neurons across all iterations.

Average response profile (Figure 3)

To calculate the average response profile of cells, we first determined if the response to a given condition was inhibitory or excitatory by using a paired t-test across trials comparing the response to baseline. Subsequently, to average across response profiles of neurons with different firing rates we normalized the PSTH of each neuron, in a fashion similar to Fogassi and colleagues [4]. Excitatory responses were

normalized by subtracting the average response during baseline (−1000 to 0ms relative to trial onset), and dividing by the maximum firing rate of the response (bin size = 200ms). Inhibitory responses were normalized by removing the average response during baseline and dividing by the absolute value of the minimum of the response. In this manner, the excitatory response of each neuron ranges between 0 and +1, whereas the inhibitory response of each neuron ranges between 0 and −1. Significant differences between the temporal response profile during action-execution and action-observation were assessed using a two-tailed t-test and a significance level of 0.05 (asterisks in figure). In the case of the control condition, we assessed significant difference from zero.

Congruency of response (supplementary Table 2)

All action observation/execution matching neurons displayed significant deviation from baseline firing rate during the observation and execution of the matched action (and not during the corresponding control condition). However, a more stringent criterion of response matching selectivity, is that the response amplitude for the matched action (during both observation and execution) is also statistically different than the response amplitude for other actions. We defined Broadly congruent cells (B) as cells whose response amplitude to the matched action with one effector (e.g. hand) was statistically higher than the response amplitude of both actions of the other effector (e.g. face) during both observation and execution. Matching cells (M) were defined as cells having significant response amplitude relative to baseline but not compared to actions with the other effector during both execution and observation.

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